

Sequence Listing

<110> Pharm. Co., Ltd.

<120> Modified human granulocyte-colony stimulating factor and process for producing same

<130> PCA00729/HMY

<160> 71

<170> KOPATIN 1.0

<210> 1

<211> 522

<212> DNA

<213> *Homo sapiens*

<220>

<221> CDS

<222> (1)..(522)

<400> 1

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Thr	Pro	Leu	Gly	Pro	Ala	Ser	Ser	Leu	Pro	Gln	Ser	Phe	Leu	Leu	Lys	
1				5					10					15		

tgc	tta	gag	caa	gtg	agg	aag	atc	cag	ggc	gat	ggc	gca	gcg	ctc	cag	96
Cys	Leu	Glu	Gln	Val	Arg	Lys	Ile	Gln	Gly	Asp	Gly	Ala	Ala	Leu	Gln	
		20						25					30			

gag	aag	ctg	tgt	gcc	acc	tac	aag	ctg	tgc	cac	ccc	gag	gag	ctg	gtg	144
Glu	Lys	Leu	Cys	Ala	Thr	Tyr	Lys	Leu	Cys	His	Pro	Glu	Glu	Leu	Val	
		35					40					45				

ctg	ctc	gga	cac	tct	ctg	ggc	atc	ccc	tgg	gct	ccc	ctg	agc	tcc	tgc	192
Leu	Leu	Gly	His	Ser	Leu	Gly	Ile	Pro	Trp	Ala	Pro	Leu	Ser	Ser	Cys	
		50				55					60					

ccc	agc	cag	gcc	ctg	cag	ctg	gca	ggc	tgc	ttg	agc	caa	ctc	cat	agc	240
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser
 65 70 75 80

ggc ctt ttc ctc tac cag ggg ctc ctg cag gcc ctg gaa ggg ata tcc 288
 Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser
 85 90 95

ccc gag ttg ggt ccc acc ttg gac aca ctg cag ctg gac gtc gcc gac 336
 Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp
 100 105 110

ttt gcc acc acc atc tgg cag cag atg gaa gaa ctg gga atg gcc cct 384
 Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro
 115 120 125

gcc ctg cag ccc acc cag ggt gcc atg ccg gcc ttc gcc tct gct ttc 432
 Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe
 130 135 140

cag cgc cgg gca gga ggg gtc ctg gtt gct agc cat ctg cag agc ttc 480
 Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser Phe
 145 150 155 160

ctg gag gtg tcg tac cgc gtt cta cgc cac ctt gcg cag ccc 522
 Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
 165 170

<210> 2

<211> 174

<212> PRT

<213> *Homo sapiens*

<400> 2

Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
 1 5 10 15

Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
 20 25 30

Glu Lys Leu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu Glu Leu Val
 35 40 45
 Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser Ser Cys
 50 55 60
 Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln Leu His Ser
 65 70 75 80
 Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile Ser
 85 90 95
 Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp Val Ala Asp
 100 105 110
 Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro
 115 120 125
 Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala Ser Ala Phe
 130 135 140
 Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu Gln Ser Phe
 145 150 155 160
 Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln Pro
 165 170

<210> 3

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer for the N-terminal of hG-CSF

<400> 3

cgccgccata tgacaccctt gggccctgcc ag

<210> 4
 <211> 36
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Oligonucleotide primer for the C-terminal of hG-CSF

<400> 4
 accgaattcg gatcctcagg gctgcgcaag gtggcg 36

<210> 5
 <211> 72
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Oligonucleotide for preparing *E. coli* enterotoxin II signal peptide

<400> 5
 tcatgaaaaa gaatatcgca tttcttcttg catctatgtt cgttttttct attgctacaa 60
 atgcctacgc gt 72

<210> 6
 <211> 72
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Oligonucleotide for preparing *E. coli* enterotoxin II signal peptide

<400> 6
 acgcgtaggc attttagca atagaaaaaa cgaacataga tgcaagaaga aatgcgatat 60

tctttttcat ga

72

<210> 7

<211> 39

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer coding for the N-terminal of [Ser1]hG-CSF

<400> 7

acaaatgcct acgcgtctcc cctgggccct gccagctcc

39

<210> 8

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer coding for the C-terminal of [Ser1]hG-CSF

<400> 8

accgaattcg gatactcagg gctgcgcaag gtggcgtaga ac

42

<210> 9

<211> 65

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer coding for *E.coli* enterotoxin II Shine-Dalgarno sequence

<400> 9

cggtttcct ctagagggtg aggtgtttta tgaaaaagaa tatcgcatTT cttcttgcAT 60

ctatg

65

<210> 10

<211> 45

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide containing BamHI restriction site

<400> 10

accgaattcg gatcctcagg gctgcgcaag gtggcgtaga acgcg

45

<210> 11

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Last five amino acids of *E. coli* enterotoxin II signal peptide plus the 1st to the 5th amino acids of hG-CSF

<400> 11

Thr Asn Ala Tyr Ala Thr Pro Leu Gly Pro

1

5

10

<210> 12

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide for preparing [Thr1]hG-CSF

<400> 12

acaaatgcct acgcgacacc cctgggccct

30

<210> 13
 <211> 30
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Antisense of SEQ ID NO: 12
 <400> 13
 agggcccagg ggtgtcgcgt aggcatttgt

30

<210> 14
 <211> 8
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> N-terminal sequence of *E. coli* enterotoxin II signal peptide
 having threonine as the 4th amino acid

<400> 14
 Met Lys Lys Thr Ile Ala Phe Leu
 1 5

<210> 15
 <211> 33
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Oligonucleotide for substituting the 4th amino acid of *E. coli*
 enterotoxin II signal peptide with threonine

<400> 15
 ggtgttttat gaaaaagaca atcgcat ttc

33

<210> 16
 <211> 33
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Antisense of SEQ ID No: 15

<400> 16
 gaagaaatgc gattgtcttt ttcataaaac acc

33

<210> 17
 <211> 8
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> C-terminal sequence of *E. coli* enterotoxin II signal peptide
 having glutamine as the 22nd amino acid

<400> 17
 Asn Ala Gln Ala Thr Pro Leu Gly
 1 5

<210> 18
 <211> 26
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Oligonucleotide for substituting the 22nd amino acid of *E. coli* enterotoxin II signal peptide with glutamine

<400> 18
 caaatgccca agcgacaccc ctgggc

26

<210> 19

<211> 26
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Antisense of SEQ ID NO: 18

<400> 19
 gccacaggggt gtcgcttggg catttg 26

<210> 20
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Oligonucleotide for modifying *E. coli* enterotoxin II Shine-Dalgarno sequence
 <400> 20
 tctagagggtt gaggtgtttt atga 24

<210> 21
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Antisense of SEQ ID NO: 20

<400> 21
 tcataaaaca cctcaacctc taga 24

<210> 22
 <211> 66
 <212> DNA
 <213> Artificial Sequence

<220>

<223> S1 oligomer having *E. coli*-preferred nucleotide sequence coding for the 6th to 26th amino acids of [Ser17]hG-CSF

<400> 22

cagcctcttc tcttcacaa tctttccttc ttaagtctct tgaacaagtt agaaagatcc 60

aaggcg

66

<210> 23

<211> 66

<212> DNA

<213> Artificial Sequence

<220>

<223> Antisense of SEQ ID NO: 22 (AS1 oligomer)

<400> 23

ccgggtcgga gaagagaagg tgtagaaaag gaagaattca gagaacttgt tcaatctttc 60

taggtt

66

<210> 24

<211> 21

<212> PRT

<213> *Escherichia coli*

<220>

<221> SIGNAL

<222> (1).. (21)

<223> *E. coli* OmpA signal peptide

<400> 24

Met Lys Lys Thr Ala Ile Ala Ile Ala Val Ala Leu Ala Gly Phe Ala
 1 5 10 15

Thr Val Ala Gln Ala
 20

<210> 25
 <211> 18
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Oligonucleotide containing Hind III recognition site

<400> 25
 gttgcgcaag cttctcga

18

<210> 26
 <211> 18
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Antisense of SEQ ID NO: 25

<400> 26
 tcgagaagct tgcgcaac

18

<210> 27
 <211> 39
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Oligonucleotide for the N-terminal of [Ser1] hG-CSF

<400> 27
 gttgcgcaag cttctcccct gggccctgcc agctccctg

39

<210> 28
 <211> 39

<212> DNA
 <213> Artificial Sequence

<220>
 <223> Oligonucleotide containing EcoRI restriction site

<400> 28
 accgaattct cagggctgcg caaggtggcg tagaacgcg

39

<210> 29
 <211> 13
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> *E. coli* OmpA signal peptide plus the 1st to the 5th amino acids of [Ser1]hG-CSF

<400> 29
 Gly Phe Ala Thr Val Ala Gln Ala Ser Pro Leu Gly Pro
 1 5 10

<210> 30
 <211> 30
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Oligonucleotide for preparing [Thr1]hG-CSF

<400> 30
 accgttgccg aagctacacc cctgggccct

30

<210> 31
 <211> 30
 <212> DNA
 <213> Artificial Sequence

<220>

<223> Antisense of SEQ ID NO: 30

<400> 31

agggccccagg ggtgtagctt gcgcaacggt

30

<210> 32

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide for preparing [Ser17]hG-CSF

<400> 32

agcttcctgc tcaagtcttt agagcaagtg agg

33

<210> 33

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Antisense of SEQ ID NO: 32

<400> 33

cctcacttgc tctaaagact tgagcaggaa gct

33

<210> 34

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide for preparing [Thr17]hG-CSF

<400> 34 33
agcttcctgc tcaagacctt agagcaagtg agg

<210> 35
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense of SEQ ID NO: 34

<400> 35 33
cctcacttgc tctaaggtct tgagcaggaa gct

<210> 36
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide for preparing [Ala17]hG-CSF

<400> 36 33
agcttcctgc tcaaggcctt agagcaagtg agg

<210> 37
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Antisense of SEQ ID NO: 36

<400> 37 33
cctcacttgc tctaaggcct tgagcaggaa gct

<210> 38
<211> 33
<212> DNA
<213> Artificial Sequence

<220>

<223> Oligonucleotide for preparing [Gly17]hG-CSF

<400> 38

agcttcctgc tcaaggcctt agagcaagtg agg

33

<210> 39
<211> 33
<212> DNA
<213> Artificial Sequence

<220>

<223> Antisense of SEQ ID NO: 38

<400> 39

cctcacttgc tctaagccct tgagcaggaa gct

33

<210> 40
<211> 33
<212> DNA
<213> Artificial Sequence

<220>

<223> Oligonucleotide for preparing [Asp17]hG-CSF

<400> 40

agcttcctgc tcaaggactt agagcaagtg agg

33

<210> 41
<211> 33
<212> DNA
<213> Artificial Sequence

<220>

<223> Antisense of SEQ ID NO: 40

<400> 41

cctcacttgc tctaagtcct tgagcaggaa gct

33

<210> 42

<211> 18

<212> PRT

<213> *Escherichia coli*

<220>

<221> SIGNAL

<222> (1)..(18)

<223> *E. coli* Gene III signal peptide

<400> 42

Met	Lys	Lys	Leu	Leu	Phe	Ala	Ile	Pro	Leu	Val	Val	Pro	Phe	Tyr	Ser
1				5					10					15	

His Ser

<210> 43

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide containing Nco I restriction site

<400> 43

tatagccata gcacatgga g

21

<210> 44

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Antisense of SEQ ID NO: 43

<400> 44

ctccatggtg ctatggctat a

21

<210> 45

<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> The 2nd to the 10th amino acids of hG-CSF

<400> 45

Pro Leu Gly Pro Ala Ser Ser Leu

1

5

<210> 46

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer coding for the 2nd to the 10th amino acids of hG-CSF plus an additional cytosine at its 5'-end

<400> 46

ccccctgggc cctgccagct ccctg

25

<210> 47

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> Antisense of SEQ ID NO: 46

<400> 47

caggagctg gcaggccca ggggg

25

<210> 48

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> *E. coli* Gene III signal peptide plus the 1st to the 5th amino acids of hG-CSF

<400> 48

Phe	Tyr	Ser	His	Ser	Thr	Pro	Leu	Gly	Pro
1				5					10

<210> 49

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> The 1st to the 9th amino acids of [Met2,Val3]hG-CSF

<400> 49

Thr	Met	Val	Gly	Pro	Ala	Ser	Ser	Leu
1				5				

<210> 50

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide for preparing [Met2,Val3]hG-CSF

<400> 50

tacggtcca tggtagggccc tgccagctcc ctg

33

<210> 51

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Antisense of SEQ ID NO: 50

<400> 51

caggagctg gcagggccca ccatggacgc gta

33

<210> 52

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> *E. coli* Gene III signal peptide plus the 1st to the 5th amino acids of [Met2,Val3]hG-CSF

<400> 52

Phe Tyr Ser His Ser Thr Met Val Gly Pro

1

5

10

<210> 53

<211> 23

<212> PRT

<213> *Escherichia coli*

<220>

<221> SIGNAL

<222> (1)..(23)

<223> Thermoresistant *E. coli* enterotoxin II signal peptide

<400> 53

Met Lys Lys Asn Ile Ala Phe Leu Leu Ala Ser Met Phe Val Phe Ser
 1 5 10 15

Ile Ala Thr Asn Ala Tyr Ala
 20

<210> 54

<211> 23

<212> PRT

<213> Artificial Sequence

<220>

<223> Modified thermoresistant *E. coli* enterotoxin II signal peptide

<400> 54

Met Lys Lys Thr Ile Ala Phe Leu Leu Ala Ser Met Phe Val Phe Ser
 1 5 10 15

Ile Ala Thr Asn Ala Gln Ala
 20

<210> 55

<211> 96

<212> DNA

<213> Artificial Sequence

<220>

<223> Nucleotide sequence coding for the 1st to 32nd amino acids of [Ser1, Ser17]hG-CSF

<220>

<221> CDS

<222> (1)..(96)

<400> 55
 tct ccc ctg ggc cct gcc agc tcc ctg ccc cag agc ttc ctg ctc aag 48
 Ser Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
 1 5 10 15

tct tta gag caa gtg agg aag atc cag ggc gat ggc gca gcg ctc cag 96
 Ser Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
 20 25 30

<210> 56
 <211> 32
 <212> PRT
 <213> Artificial Sequence

<400> 56
 Ser Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
 1 5 10 15
 Ser Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
 20 25 30

<210> 57
 <211> 96
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Nucleotide sequence coding for the 1st to the 32nd amino acids of [Ser1]hG-CSF

<220>
 <221> CDS
 <222> (1)..(96)

<400> 57
 tct ccc ctg ggc cct gcc agc tcc ctg ccc cag agc ttc ctg ctc aag 48
 Ser Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
 1 5 10 15

tgc tta gag caa gtg agg aag atc cag ggc gat ggc gca gcg ctc cag 96
 Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
 20 25 30

<210> 58
 <211> 32
 <212> PRT
 <213> Artificial Sequence

<400> 58
 Ser Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
 1 5 10 15
 Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
 20 25 30

<210> 59
 <211> 96
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Nucleotide sequence coding for the 1st to the 32nd amino acids of [Ser17]hG-CSF

<220>
 <221> CDS
 <222> (1)..(96)

<400> 59 48
 aca ccc ctg ggc cct gcc agc tcc ctg ccc cag agc ttc ctg ctc aag
 Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
 1 5 10 15
 tct tta gag caa gtg agg aag atc cag ggc gat ggc gca gcg ctc cag 96
 Ser Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
 20 25 30

<210> 60
 <211> 32
 <212> PRT
 <213> Artificial Sequence

<400> 60
 Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
 1 5 10 15
 Ser Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
 20 25 30

<210> 61
 <211> 96
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Nucleotide sequence coding for the 1st to the 32nd amino acids of [Thr17]hG--CSF

<220>
 <221> CDS
 <222> (1)..(96)

<400> 61
 aca ccc ctg ggc cct gcc agc tcc ctg ccc cag agc ttc ctg ctc aag 48
 Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
 1 5 10 15
 acc tta gag caa gtg agg aag atc cag ggc gat ggc gca gcg ctc cag 96
 Thr Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
 20 25 30

<210> 62
 <211> 32

<212> PRT

<213> Artificial Sequence

<400> 62

Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
 1 5 10 15

Thr Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
 20 25 30

<210> 63

<211> 96

<212> DNA

<213> Artificial Sequence

<220>

<223> Nucleotide sequence coding for the 1st to the 32nd amino acids of [Ala17]hG-CSF

<220>

<221> CDS

<222> (1)..(96)

<400> 63

aca ccc ctg ggc cct gcc agc tcc ctg ccc cag agc ttc ctg ctc aag 48
 Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
 1 5 10 15

gcc tta gag caa gtg agg aag atc cag ggc gat ggc gca gcg ctc cag 96
 Ala Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
 20 25 30

<210> 64

<211> 32

<212> PRT

<213> Artificial Sequence

<400> 64

25

Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
1 5 10 15

Ala Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
20 25 30

<210> 65
<211> 96
<212> DNA
<213> Artificial Sequence

<220>
<223> Nucleotide sequence coding for the 1st to the 32th amino acids of [Gly17]hG-CSF

<220>
<221> CDS
<222> (1)..(96)

<400> 65 48
aca ccc ctg ggc cct gcc agc tcc ctg ccc cag agc ttc ctg ctc aag
Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
1 5 10 15
ggc tta gag caa gtg agg aag atc cag ggc gat ggc gca gcg ctc cag 96
Gly Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
20 25 30

<210> 66
<211> 32
<212> PRT
<213> Artificial Sequence

<400> 66
Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
1 5 10 15

Gly Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln

20 25 26 30

<210> 67
 <211> 96
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Nucleotide sequence coding for the 1st to the 32nd amino acids of [Met2, Val3]hG-CSF

<220>
 <221> CDS
 <222> (1)..(96)

<400> 67 48
 aca atg gtc ggc cct gcc agc tcc ctg ccc cag agc ttc ctg ctc aag
 Thr Met Val Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
 1 5 10 15
 tgc tta gag caa gtg agg aag atc cag ggc gat ggc gca gcg ctc cag 96
 Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
 20 25 30

<210> 68
 <211> 32
 <212> PRT
 <213> Artificial Sequence

<400> 68
 Thr Met Val Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
 1 5 10 15
 Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
 20 25 30

<210> 69

<211> 96
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Nucleotide sequence coding for the 1st to the 32nd amino acids of [Met2, Val3, Ser17]hG-CSF

<220>
 <221> CDS
 <222> (1)..(96)

<400> 69
 aca atg gtc ggc cct gcc agc tcc ctg ccc cag agc ttc ctg ctc aag 48
 Thr Met Val Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys
 1 5 10 15

tct tta gag caa gtg agg aag atc cag ggc gat ggc gca gcg ctc cag 96
 Ser Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
 20 25 30

<210> 70
 <211> 32
 <212> PRT
 <213> Artificial Sequence

<400> 70
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Ser Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
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 <211> 10
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 <213> Artificial Sequence

<220>

<223> Modified Shine-Dalgarno sequence

<400> 71

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